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The Use of Biofilm Dipping Reactor for Citric Acid Production

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A new type bioreactor (biofilm dipping reactor), which is suitable for the good strain in the surface culture was developed. The citric acid productivity of the biofilm dipping reactor was slightly higher than that of the surface culture. The citric acid production rate of a biofilm dipping reactor was almost constant in the dipping period range of 6-20s, and decreased with the dipping period over 20s. The biofilm dipping reactor was useful for the strain that is suitable for the surface culture.

Key Words : Citric Acid, Biofilm, *Aspergillus niger*, Surface Culture, Dipping Period

1. Introduction

Aerobic fermentation has been performed using a liquid surface culture, solid surface culture, and submerged culture. The productivity of the surface culture is generally low compared with the submerged culture. Therefore, most of the useful materials are now produced by the submerged culture [1]. However, several strains used in the surface culture are not suitable for the submerged culture [2]. It is difficult to improve the productivity of these strains by the conventional cultivation method. In addition, the surface culture has been carried out in food industry such as acetic acid fermentation, because the products by the surface culture have superior flavor compared with those by the usual submerged culture. We therefore developed a new type bioreactor (biofilm dipping reactor) that is suitable for the good strain in the surface culture. A culture condition similar to the surface culture is performed by the biofilm dipping reactor (BDR), because the BDR is able to control the exposure period to the air and dipping period in the liquid medium of the biofilm. Furthermore, the BDR has advantages in product separation, control of fermentation such as oxygen supply [3], and long term operation such as continuous and repeated batch cultures [4], because the biomass is separated as the biofilm from the liquid medium.

In this study, we evaluated the citric acid productivity of the BDR compared with the surface culture. The effect of the dipping time of the biofilm on citric acid production was also examined.

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2. Materials and Methods

2. 1 Strain and medium

Aspergillus niger Yang No. 2, a good strain for citric acid production using the semi-solid culture [2, 5], was used for this study. Before the experiment, the spores were precultured on an agar slant at 28 ℃ for 7-15 d. The liquid medium used for the citric acid production was composed of 140 g/*l* sucrose, 10 g/*l* KH_2PO_4, 2.0 g/*l* NH_4NO_3, 250 mg/*l* $MgSO_4 \cdot 7H_2O$, 14 mg/*l* $MnSO_4 \cdot 5H_2O$, 21 mg/*l* $FeCl_3 \cdot 6H_2O$, and deionized water.

2.2 Biofilm dipping reactor

A schematic diagram of the biofilm dipping reactor (BDR) is shown in Fig. 1. The reactor was a horizontal glass cylinder, 20 cm in length, 15 cm in diameter and about 3.5 *l* in volume. Inside the BDR, segment shaped stainless steel meshes which were 1 mm in thickness, 7.4 cm in radius, were supported by a shaft and rotated aseptically by a stepping motor using magnetic coupling. The mesh opening and the wire diameter were 1.5 mm and 0.35 mm, respectively. The stepping motor was controlled by a sequence controller. The biofilm attached on the segment shaped mesh stayed mutually in the gas phase and liquid phase for set periods as shown in Fig. 2. Air was supplied into the gas phase of the BDR by an air compressor after being passed through an air filter and a humidifier. The BDR system was set in a constant temperature chamber kept at 30 ℃.

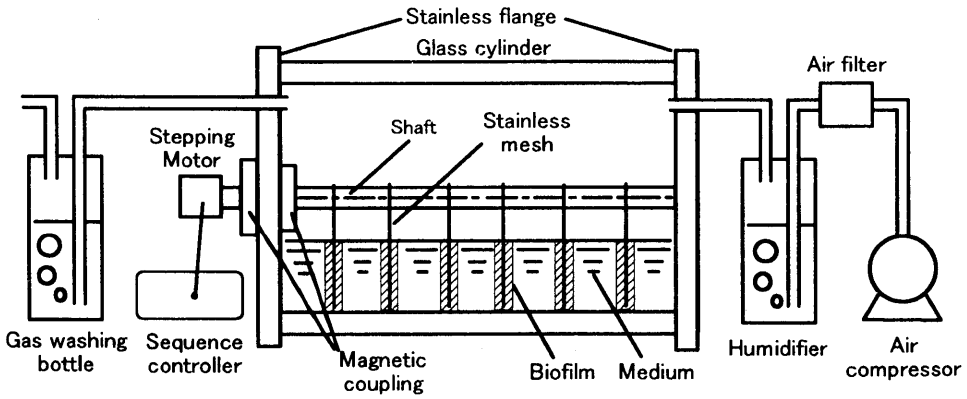

Fig. 1 Schematic diagram of biofilm dipping reactor (BDR).

2.3 Culture by BDR

The cultivation was started by inoculating the spore suspension into the BDR. The pH of the medium was adjusted to 4.15 at the start of the cultivation, but thereafter, it was not controlled during the cultivation. The experimental range was as follows: air flow rate, 0.375 vvm; medium volume, 500 m*l*; number of mesh, 24. The segment shaped meshes were continuously rotated at 20 rpm during the first 3 d of cultivation, but thereafter it was controlled at 9 s in the gas phase and 6-120 s in the liquid phase (Fig. 2).

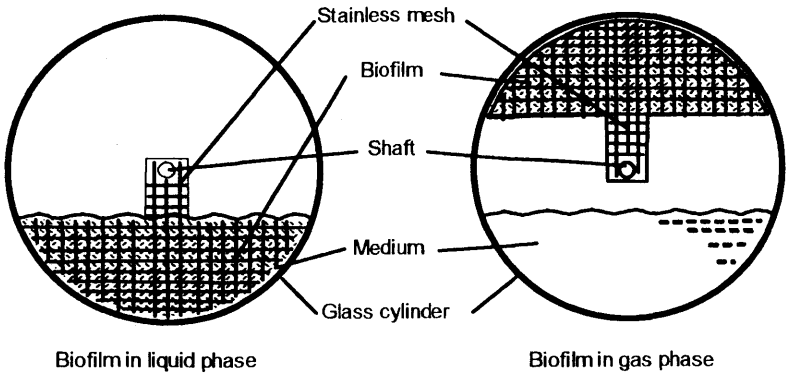

Fig. 2 Schematic of biofilm during cultivation.

2.4 Surface culture

A 500 m*l*-large mouth bottle, 8.4 cm in diameter, 13.5 cm in height, and 4.5 cm in bottleneck diameter was used for the surface culture. Air was supplied into the gas phase of the culture bottle in the same way as the BDR. The medium volume was 200 m*l*. The medium beneath the biofilm was gently agitated for 5 min everyday after 3 d of cultivation. The other cultivation conditions were the same as that of the BDR.

2.5 Assays

A 2.5 m*l* aliquot of culture broth was sampled at regular intervals and filtered using a membrane filter (pore size, 0.45μm). The filtrate was analyzed for citric acid and sugar. The citric acid and sugar were analyzed by HPLC with a Yanapak column (SAX-801) and a Shodex column (KS-801), respectively. The dissolved oxygen concentration in the medium was measured by an oxygen electrode.

3. Results and Discussion

3.1 Time course of citric acid production

The typical time course of citric acid production by the BDR was compared with that of the surface culture (Fig. 3). The upper surface of the biofilm in surface culture began to sporulate at 3 d of cultivation, but the biofilm of the BDR scarcely sporulated during the cultivation. The maximum citric acid concentration for the BDR was on a level with the surface culture. The production rate of the citric acid for the BDR was slightly higher than that for the surface culture. However, the cultivation time for the BDR at which vigorous citric acid production started was short compared with that for the surface culture (ex. about 3 d for BDR and 7 d for surface culture). From these results, BDR is an excellent reactor for a strain that is suitable for surface culture.

3.2 Effect of dipping period on citric acid production

The citric acid production rate was almost constant in the 6-20 s of dipping period range, but it drastically decreased over 20 s as shown in Fig. 4. This may be due to the oxygen deficiency in the liquid phase.

Dissolved oxygen concentrations in the trough medium dropped to 3.0-3.2 mg/l for the dipping period of 6 s, 1.3-1.5 mg/l for 20 s and 0.8-0.9 mg/l for 80 s. Although the difference in the dissolved oxygen concentration between 20 s and 80 s is too small, a critical value of dissolved oxygen concentration for citric acid production may exist in the range of 0.9-1.3 mg/l. In order to obtain a high citric acid production rate, it should be noted that the dipping period in the range below 20 s is preferable.

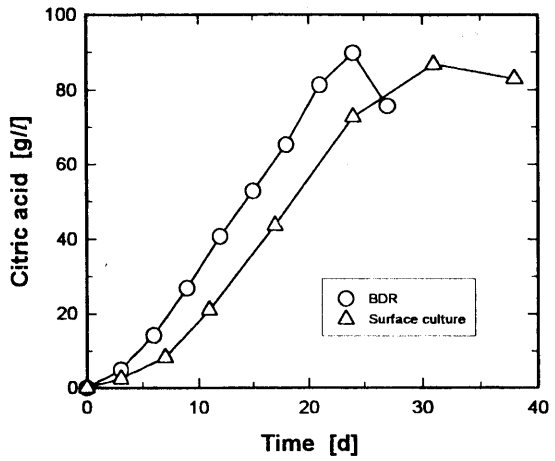

Fig. 3 Time courses of citric acid production for the biofilm dipping reactor and surface culture.

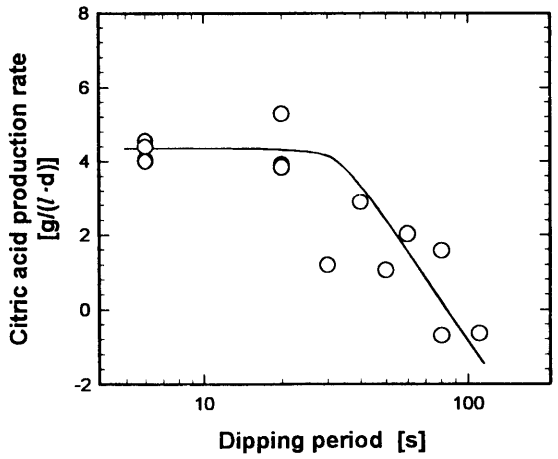

Fig. 4 Effect of dipping period on citric acid production rate.

4. Conclusion

The BDR was useful for citric acid production by the strain that is suitable for surface culture. This reactor will be also useful in other fermentation field such as the food industry, in which the surface culture have been performed despite the low productivity because of its superior flavor.

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